A
Synopsis of thesis entitled

Biosynthesis of gold nanoparticles & its anti-*Candida albicans* activities

to be submitted for registration of
Ph.D. in Biotechnology
Under the
Faculty of Science

To
Swami Ramanand Teerth Marathwada University,
Nanded

By
Kolhe Ramesh Dnyaneshwar
Research scholar

Under guidance of
Prof. R. M. Mulani
Prof. of Botany, School of Life Sciences,
& Registrar
Swami Ramanand Teerth Marathwad university,
Nanded.

May 2019

1) Introduction:

The word “nano” originated from “nano' which means extremely small (one billionth part 10^{-9}) (Alanazi *et al.*, 2010). Gold nanoparticles possess very strong optical absorption and scattering at visible and near-infrared wavelengths, so they are used in chemical and biological sensing, imaging, drug delivery and photothermal therapies (Demers *et al*., 2017). Biosynthesis of gold nanoparticles is eco -friendly and without the use of toxic, chemicals and low maintenance (Ahmad *et al*., 2013, Huang *et al*., 2007 & Parveen *et al*., 2016

Biosynthesis of gold nanoparticles are done using various plant extracts-like, *Citrus limon* Burm, *Citrus reticulata* Blanco, *Citrus sinensis* Osbeck (Sujitha & Kannan 2013), *Rosa hybrid* (Noruzi *et al*., 2011), *Murraya* koenigi (Philip *et al*., 2011), *Artocarpus heterophyllus* Lam.(Basavegowda *et al*., 2015), *Zingiber officinale* Roscoe (Velmurugan *et al* ., 2014), *Benincasa hispida* (Aromal & Philip 2012), *Pisona grandis* (Firdhouse *et al*., 2014) and *Elettaria cardamomum* Matan (Rajan *et al*., 2017). Das *et al* (2015) reported the biological synthesis of GNPs using bark extracts of *Abroma augusta* L Taxo and suggested that, polyphenols present in the bark extract acts as reducing and stabilising agent in the synthesis of GNPs.

Nanomaterials are well known for their antimicrobial efficacy. The antibacterial activities of gold nanoparticles are widely reported. GNPs have been shown to inhibit growth of various bacteria including the human pathogenic bacteria like *Staphylococcus* sp, *Bacillus* sp (Velmurugan *et al*., 2014). Gold nanoparticles has been reported to inhibit the growth of filamentous fungi as well as yeasts. GNPs considerably inhibited, adhesion, and developing biofilm. These are considered as important virulence factors in *C. albicans* (Yu et al. (2016). A recent study reported the effect of gold nanoparticles on the biofilms of *C. albicans*. They have reported an MIC value of 63.38 ppm against biofilms of *C. albicans* (Yu *et al*., 2016).

2) Objectives:

1. To study biological synthesis of gold nanoparticles.

2. To characterize biologically synthesized gold nanoparticles

4. To study the cytotoxicity of gold nanoparticles

3. To study the effect of gold nanoparticles on *C. albicans.*

4. Establishment of MIC of gold nanoparticles against *C. albicans*

6. To study the effect of gold nanoparticles against Planktonic and biofilm form of *C. albicans*

3) Review of literature:

Biosynthesis of gold nanoparticles is eco -friendly and without the use of toxic, chemicals and low maintenance (Ahmad *et al*., 2013, Huang *et al*., 2007 & Parveen *et al*., 2016). The plant extracts contain sugars, terpenoids, polyphenols, alkaloids, phenolic acids and proteins etc. Which may act as capping agent as well as reducing agents (Parveen *et al* ., 2016). Biosynthesis of nanoparticles can be done by using the extracts of plants, algae (Govindaraju *et al*., 2008), diatoms (Scarano and Morelli 2002; Lengke *et al*.,2007), bacteria (Kowshik *et al*., 2002), yeast (Rautaray *et al*., 2003)

Wani & Ahmad (2013) applied Sonochemical method for the synthesis of gold nanoparticles. Nanodiscs of 25 nm size were obtained by tin chloride. Sodium borohydride produced polyhedral nanoparticles having the average size 30 nm. The MIC of 25 nm gold nanodiscs against the *Candia albicans* ATCC 90028 and clinical isolate were 16 ug/ml and 32 ug/ml respectively, while for 30 nm polyhedral gold nanoparticles 32 ug/ml and 128 ug/ml respectively. This indicated that gold nanoparticles show size and shape dependant antifungal activity against *C. albicans*. Small sized gold nanoparticles exhibited more fungicidal activity compared to larger gold nanoparticles. The possible mechanism of

action involves the inhibition of H+ ATPase which leads to intracellular acidification and cell death.

Shankar *et al*.,(2014) reported the synthesis of gold, gold-silver nanoparticles and silver nanoparticles by using the fruit peel extract of *Lansium domesticum*. They showed that gold-silver nanoparticles and silver nanoparticles have antimicrobial activity. 16 ug/ml and 8 ug/ml of silver nanoparticles inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*. MIC of GNP against *S. aureus* and *E. coli* was 16 ug /ml.

GNPs synthesized from *Vetiveria zizanioides* (Linn.) Nash root and *Cannabis sativa* leaf extracts was tested for its antifungal activity against fungal pathogens *Penicillium* sp, *Mucor* sp, *Fusarium* sp *&* *Aspergillus* sp. Green synthesized GNPs possessed effective antifungal activity compared to chemicaly synthesized nanoparticles. Gold nanoparticles killed the fungi. As the size of particles decreased the fungicidal activity was increased because small size particle might penetrate cell membrane more easily and compared to larger one (Swain *et al*., 2016). Similar antifungal activity was reported by Ahmad *et al*.,(2013).

Aspergillus flavus, Aspergillus niger, and *C. albicans* were inhibited by gold nanoparticles which was produced by the aqueous seed extract of *Abelmoschus esculentus* W and A. Size of gold nanoparticle was in the range of 45–75 nm. The maximum zone of inhibition was observed in the GNPs against *Puccinia graminis* (17 mm) and *C. albicans* (18 mm). The antifungal effect of gold nanoparticles at 50 µl was determined by the zone of inhibition. The synthesized GNPs displayed antifungal against *Puccinia graminis* (17 mm), *Aspergillus flavus* (16 mm), *Aspergillus niger* (15 mm) and *C. albicans* (18 mm). GNPs conformed as antifungal agent and capable of antifungal rendering efficacy used against fungal infection (Jayaseelan *et al*., 2013). Gold nanoparticles associated with photosensitizes like methylene blue (MB) or toluidine blue O (TB) acts as the inhibitor of *C. albicans* biofilm. The nanoparticles killed *C. albicans* and inhibited hyphal of biofilm in a mouse model (Sherwani *et al*., 2015).

Biofilm formation and invasion into the host cells are the important steps is infection. *C. albicans* and *Pseudomonas aeruginosa* formed pathogenic biofilm and invaded to dental pulp stem cell which was inhibited by the gold nanoparticles (Bernardis *et al*., 2001).

Gold nanoparticles are produced by apple extract (10-20 nm) and trisodium citrate. This particle did not inhibit growth and hyphae formation of *C. albicans*. RT–PCR showed that there was the normal expression of genes such as, HWP1, LEC1, and ALS3. But it inhibits the biofilm formation of *C. albicans* which was confirmed by MTT assay. Less then 20 ppm gold nanoparticles decreased 80 % of biofilm. The biofilm density was reduced after the treatment of gold nanoparticles. During the formation of biofilm in *C. albicans*, the first step is adhesion of the fungal cells to the substrate surfaces. Nanoparticles did not allow adhesion to attached to the bottom of the surface due to which planktonic growth increased but adhesion of the fungal cell to the substrate was decreased. The IC50 value gold nanoparticles against the *Pseudomonas aeruginosa* was 68.56 -75.01 ppm. Gold nanoparticles also inhibited the invasion of *C. albicans* to host dental pulp stem cell and the IC50 against the C. *albicans* was 63.38 ppm. *C. albicans* interacts with the receptor that generate the immune response in dental pulp stem cell due to the expression of INF-α and some immune factor. *C. albicans* cell and gold nanoparticles were attracted to each other electrostatically. This interaction may be due to the carbonyl group present on the gold nanoparticles. It has negative Zeta potential (− 9.39 mV) (Yu *et al*., 2016; Gantner *et al*., 2003).

Biofilm formation by *C. albicans* are mediated quorum sensing molecules by cell to cell signaling molecules which are inhibited by farnesol, (S)-4,5 Dihydoxypentane-2,3-dione [(S)-DPD] and carvacrol (Darouiche 2004; Parsek & Greenberg 2005). Naglik *et al*.,(2003) reported that *C. albicans* and other pathogens that secret extracellular hydrolytic enzymes secreted aspartyl proteinases, phospholipases, and lipases which damages and invading the host cells. Jebali *et al*., (2014) reported that naked triangular gold nanoparticles and peptide ligands associated with triangular gold nanoparticles show anti-*Candida* activity.

4) Material and methods:

4.1) Chemicals:

Gold(III) chloride trihydrate, 2, 3-bis (2- methoxy-4-nitro-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT), menadione from the Sigma-Aldrich Chem. Ltd. Mumbai, India. Methanol, DMSO, yeast extract, peptone, dextrose, nutrient broth, mannitol, K_2PO_4, etc.

4.2) Procedure:

The bark will chopped into pieces and shade dried for 10 to 20 days. 20 gm of the bark powder will be extracted in 250 ml of solvent with increasing polarity *viz.* Petroleum ether > chloroform > Methanol and distilled water at their boiling temperature using a Soxhlet's apparatus, for 2-3 hrs. The extract will be dried at room temperature. This powder will be stored at 4^0C for the synthesis of gold nanoparticles (Bansode *et al.*, 2016).

The leaves of *Polyalthia longifolia* B and H - False will be thoroughly washed with distilled water and can be blot dried with tissue paper. After drying the leaves will be chopped into small pieces. Ten grams of chopped leaves will be added in 100 ml of distilled water and boiled for five minutes to make an aqueous extract. The aqueous extract will be filtered through a 0.45 µM membrane filter and used.(Halbandge *et al.,* 2017)

The gold nanoparticles will be characterized by UV – Visible spectophotomete, XRD, EDX, SEM TEM , particle size analysis, etc

4.3) Culture of *Candida albicans:*

Authentic culture of *C. albicans*, ATCC 90028 will be obtained from the Institute of Microbial Technology, Chandigarh, India. Clinical isolate (GMC-3) will be obtained from Gramin medical college, Nanded, Maharashtra state, India. Both the cultures will be maintained on Yeast-Peptone-Dextrose (YPD, 1:2:2 %) agar slant at 4^0C (Raut *et al.*, 2013)

4.4) Planktonic Growth of *Candida albicans:*

Effect BME-AL-GNP of on the growth of planktonic cells of *C. albicans* will be studied by using the standard broth micro dilution method as per Clinical and Laboratory Standards Institute Guidelines (CLSI, 2008) with slight modification in which spider medium will be used instead of RPMI). 1×103 cells/ml will be added (Raut *et al*., 2013)

4.5) Adhesion Assay of *Candida albicans:*

Effect of BME-AL-GNP on adherence of *C. albicans* to polystyrene will be investigated by utilizing micro plate based assay (Raut *et al*., 2013).

4.6) Biofilm Development of *Candida albicans:*

C. albicans biofilms will be developed on polystyrene surface area of 96 well plates as per standard methodologies (Raut et al., 2013). 100 µl of 1×107 cells/ml cell suspension will be inoculated in PBS and plates shall be incubated at 37°C for 90 min to permit attachment of cells on the polystyrene surface (Raut *et al.*, 2013).

4.7) Evaluation of biofilm by metabolic assay:

Biofilm growth was evaluated using XTT. The XTT solution will be prepared by mixing 1 mg/ml XTT salt in PBS and stored at -20°C.before to use (Raut *et al*., 2013)

4.8) Cell surface hydrophobicity (CSH) of *Candida albicans:*

C. albicans cells will be resuspended in PBS and diluted to set an optical density (OD) of 0.5 absorbance at 620 nm wavelength. From this suspension 1.3 ml will be pipetted into each of three borosilicate glass tubes. From each tube, 100 ul of the cell will be pipetted into wells of a 96 well microtiter plate.

The percentage hydrophobicity of the cell surface will be calculated by the formula percentage of CSH = (1-final OD of aqueous phase/ initial OD of cell suspension) X 100 (Raut *et al*., 2010).

4.9) Hemolytic assay of blood:

The hemolysis of blood by BME-AL-GNP will be studied using RBCs cells. Human erythrocytes from healthy persons will be collected in tubes containing EDTA (1–2 mg/ml) as an anti-coagulant. The erythrocytes will be acquired by centrifugation blood for 10 min at 2000 rpm, Percentage Hemolysis = $_{A}$450 of BME-AL-GNP sample−A450 of buffer treated sample/ A450 of 1 % Triton−X 100 sample−A450of buffer sample×100 (Rajput *et al*., 2013).

4.10) Sample preparations of *C. albicans* developing biofilm for Scanning Electron Microscopy:

Adhesion of *Candida albicans* cells on polystyrene discs (1×10^7 cells/ ml) will be carried out in 12-well plates at 37 ^{0}C at 50 rpm for 90 min. For SEM, samples will be fixed in 2.5 % of glutaraldehyde in 0.1 mol phosphate buffer (pH 7.2) for 24 h at 4 ^{0}C. Samples will be post fixed in 2 % aqueous solution of osmium tetraoxide for 4 h, then dehydrated in a series of graded alcohols. The samples will be mounted over stubs and gold coating will be performed using an automated gold coater. Images will be obtained by JOEL 6360 (Japan) scanning electron microscope (Kathwate & Karuppayil 2013).

4.11) Statistical analysis:

Statistical analysis values are the mean with standard deviations obtained from three dissimilar observations. This result compared using Student's t - test. A value of $P < 0.05$ will be considered statistically significant (Bansode *et al*., 2016).

5) Importance of study

5.1 International status:

Gold nanoparticles are produced by apple extract and trisodium citrate did not inhibit growth and hyphae formation of *C. albicans* but it inhibit the bioflim formation . Less then 20 ppm gold nanoparticles decreased 80% of bioflim. The bioflim density was reduced after the treatment of gold nanoparicles (Yu *et al.*, 2016). Gold nanoparticles coated with

indolicidin inhibited the *Candida albicans* bioflim fromation in multi-well polystyrene plate (Alteriis *et al.*, 2018)

5.2) National status:

Aspergillus flavus, Aspergillus niger, and *C. albicans* were inhibited by gold nanoparticles which was produced by the aqueous seed extract of *Abelmoschus esculentus*. Size of gold nanoparticle was in the range of 45–75 nm. The maximum zone of inhibition was observed in the GNPs against *Puccinia graminis* (17 mm) and *C. albicans* (18 mm) (Jayaseelan *et al*., 2013). Gold nanoparticles associated with photosensitizes like methylene blue (MB) or toluidine blue O (TB) acts as the inhibitor of *C. albicans* biofilm. The nanoparticles killed *C. albicans* and inhibited hyphal of biofilm in a mouse model (Sherwani *et al*., 2015).

5.3) Significance of the Study

Biosynthesis of gold nanoparticles may inhibit the *Candida albicans* which will be novel therapy against it.

6) Work plan:

Sr. No.	First year	Second Year
1	Collection of literature and Screaming of different plant for biosynthesis of gold nanoparticles	To study the effect of gold nanoparticles on *C. albicans*
2	Optimization of physical and chemical parameter for biosynthesis of gold nanoparticles	Writing the paper and thesis
3	Characterization of synthesized gold nanoparticles	If needed remaining work

.

7) References:

1. Ahmad, T., Wani, I. A., Lone, I. H., Ganguly, A., Manzoor, N., Ahmad, A & Al-Shihri, A. S. (2013). Antifungal activity of gold nanoparticles prepared by solvothermal method. *Materials Research Bulletin*, *48*(1), 12-20.
2. Alanazi, F. K., Radwan, A. A., & Alsarra, I. A. (2010). Biopharmaceutical applications of nanogold. *Saudi Pharmaceutical Journal*, *18*(4), 179-193.
3. Aromal, S. A., & Philip, D. (2012). *Benincasa hispida* seed mediated green synthesis of gold nanoparticles and its optical nonlinearity. *Physica E: Low-Dimensional Systems and Nanostructures*, *44*(7), 1329-1334.
4. Bansode, B. S., Jadhav, A. K., Vitthal, N., Ghule, D. P., Srinivas, R., Shankar, G., & Karuppayil, S. M. (2016). Analysis of phytochemical profile and antibiofilm activity of Stem Bark extract of *Terminalia arjuna* Wt & Arn against the human pathogen *Candida albicans*. *Journal of Pharmacognosy and Phytochemistry*, *5*(6), 345-356.
5. Basavegowda, N., Idhayadhulla, A., & Lee, Y. R. (2014). Phyto-synthesis of gold nanoparticles using fruit extract of *Hovenia dulcis* and their biological activities. *Industrial Crops and Products*, *52*, 745-751.
6. Bernardis, F. D., Sullivan, P. A., & Cassone, A. (2001). Aspartyl proteinases of *Candida albicans* and their role in pathogenicity. *Medical Mycology*, *39*(4), 303-313
7. Darouiche, R. O. (2004). Treatment of infections associated with surgical implants. *New England Journal of Medicine*, *350*(14), 1422-1429.
8. Das, S., Bag, B. G., & Basu, R. (2015). *Abroma augusta* Linn bark extract-mediated green synthesis of gold nanoparticles and its application in catalytic reduction. *Applied Nanoscience*, *5*(7), 867-873.
9. Demers, S. M., Hsieh, L. J., Shirazinejad, C. R., Garcia, J. L. A., Matthews, J. R., & Hafner, J. H. (2017). Ultraviolet Analysis of Gold Nanorod and Nanosphere Solutions. *The Journal of Physical Chemistry C*. 121(9), 5201-5207

10. de Alteriis, E., Maselli, V., Falanga, A., Galdiero, S., Di Lella, F. M., Gesuele, R., ... & Galdiero, E. (2018). Efficiency of gold nanoparticles coated with the antimicrobial peptide indolicidin against biofilm formation and development of Candida spp. clinical isolates. *Infection and drug resistance*, *11*, 915.
11. Firdhouse, M. J., Lalitha, P., & Sripathi, S. K. (2014). An undemanding method of synthesis of gold nanoparticles using *Pisonia grandis* (R. Br.). *Dig J Nanomater Bios*, *9*, 385-393.
12. Gantner, B. N., Simmons, R. M., Canavera, S. J., Akira, S., & Underhill, D. M. (2003). Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *The Journal of Experimental Medicine*, *197*(9), 1107-1117.
13. Govindaraju, K., Basha, S. K., Kumar, V. G., & Singaravelu, G. (2008). Silver, gold and bimetallic nanoparticles production using single-cell protein (*Spirulina platensis*) Geitler. *Journal of Materials Science*, *43*(15), 5115-5122.
14. Halbandge, S. D., Mortale, S. P., & Karuppayil, S. M. (2017). Biofabricated silver nanoparticles synergistically activate amphotericin B against mature biofilm forms of *Candida albicans*. *The Open Nanomedicine Journal*, *4*(1).
15. Huang, X., Wu, H., Liao, X., & Shi, B. (2010). One-step, size-controlled synthesis of gold nanoparticles at room temperature using plant tannin. *Green Chemistry*, *12*(3), 395-399.
16. Jayaseelan, C., Ramkumar, R., Rahuman, A. A., & Perumal, P. (2013). Green synthesis of gold nanoparticles using seed aqueous extract of *Abelmoschus esculentus* and its antifungal activity. *Industrial Crops and Products*, *45*, 423-429.
17. Jebali, A., Hajjar, F. H. E., Hekmatimoghaddam, S., Kazemi, B., Jesus, M., & Rashidi, M. (2014). Triangular gold nanoparticles conjugated with peptide ligands: a new class of inhibitor for *Candida albicans* secreted aspartyl proteinase. *Biochemical Pharmacology*, *90*(4), 349-355.
18. Kowshik, M., Deshmukh, N., Vogel, W., Urban, J., Kulkarni, S. K., & Paknikar, K. M. (2002). Microbial synthesis of semiconductor CdS nanoparticles, their characterization, and their use in the fabrication of an ideal diode. *Biotechnology and Bioengineering*, *78*(5), 583-588.

19. Kathwate, G. H., & Karuppayil, S. M. (2013). Antifungal properties of the anti-hypertensive drug: Aliskiren. *Archives of oral biology*, *58*(9), 1109-1115.
20. Lengke, M. F., Fleet, M. E., & Southam, G. (2007). Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver (I) nitrate complex. *Langmuir*, *23*(5), 2694-2699.
21. Naglik, J. R., Challacombe, S. J., & Hube, B. (2003). *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiology and Molecular Biology Reviews*, *67*(3), 400-428.
22. Noruzi, M., Zare, D., Khoshnevisan, K., & Davoodi, D. (2011). Rapid green synthesis of gold nanoparticles using *Rosa hybrida* petal extract at room temperature. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *79*(5), 1461-1465.
23. Parsek, M. R., & Greenberg, E. P. (2005). Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends in Microbiology*, *13*(1), 27-33.
24. Parveen, K., Banse, V., & Ledwani, L. (2016, April). Green synthesis of nanoparticles: Their advantages and disadvantages. In N. N. Sharma, F. L. Gaol, & J. Akhtar (Eds.), *AIP Conference Proceedings* (Vol. 1724, No. 1, p. 020048). AIP Publishing. India
25. Philip, D., Unni, C., Aromal, S. A., & Vidhu, V. K. (2011). *Murraya koenigii* leaf-assisted rapid green synthesis of silver and gold nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *78*(2), 899-904.
26. Rajan, A., Rajan, A. R., & Philip, D. (2017). *Elettaria cardamomum* seed mediated rapid synthesis of gold nanoparticles and its biological activities. *Open Nano*, *2*, 18.
27. Rajput, S. B., Shinde, R. B., Routh, M. M., & Karuppayil, S. M. (2013). Anti-*Candida* properties of asarone aldehyde of *Acorus gramineus* rhizome and three structural isomers. *Chinese Medicine*, *8*(1), 18.
28. Raut, J. S., Shinde, R. B., Chauhan, N. M., & Mohan Karuppayil, S. (2013). Terpenoids of plant origin inhibit morphogenesis, adhesion, and biofilm formation by *Candida albicans*. *Biofouling*, *29*(1), 87-96.

29. Rautaray, D., Ahmad, A., & Sastry, M. (2003). Biosynthesis of CaCO3 crystals of complex morphology using a fungus and an actinomycete. *Journal of the American Chemical Society*, *125*(48), 14656-14657.
30. Scarano, G., & Morelli, E. (2002). Characterization of cadmium-and lead-phytochelatin complexes formed in a marine microalga in response to metal exposure. *Biometals*, *15*(2), 145-151.
31. Shankar, S., Jaiswal, L., Aparna, R. S. L., & Prasad, R. G. S. V. (2014). Synthesis, characterization, in vitro biocompatibility, and antimicrobial activity of gold, silver and gold silver alloy nanoparticles prepared from *Lansium domesticum* fruit peel extract. *Materials Letters*, *137*, 75-78.
32. Sherwani, M. A., Tufail, S., Khan, A. A., & Owais, M. (2015). Gold nanoparticle-photosensitizer conjugate based photodynamic inactivation of biofilm producing cells: potential for treatment of *C. albicans* infection in BALB/c mice. *PloS one*, *10*(7), e0131684.
33. Sujitha, M. V., & Kannan, S. (2013). Green synthesis of gold nanoparticles using Citrus fruits (*Citrus limon, Citrus reticulata* and *Citrus sinensis*) aqueous extract and its characterization. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *102*, 15-23.
34. Swain, S., Barik, S. K., Behera, T., Nayak, S. K., Sahoo, S. K., Mishra, S. S., & Swain, P. (2016). Green Synthesis of Gold Nanoparticles Using Root and Leaf Extracts of *Vetiveria zizanioides* and *Cannabis sativa* and its Antifungal Activities. *BioNanoScience*, *6*(3), 205-213.
35. Velmurugan, P., Anbalagan, K., Manosathyadevan, M., Lee, K. J., Cho, M., Lee, S. M., ... & Oh, B. T. (2014). Green synthesis of silver and gold nanoparticles using *Zingiber officinale* root extract and antibacterial activity of silver nanoparticles against food pathogens. *Bioprocess and Biosystems Engineering*, *37*(10), 1935-1943.
36. Wani, I. A., & Ahmad, T. (2013). Size and shape dependant antifungal activity of gold nanoparticles: a case study of *Candida*. *Colloids and Surfaces B: Biointerfaces*, *101*, 162-170

37. Yu, Q., Li, J., Zhang, Y., Wang, Y., Liu, L., & Li, M. (2016). Inhibition of gold nanoparticles (AuNPs) on pathogenic biofilm formation and invasion to host cells. *Scientific reports*, *6*.

Signature	Signature
Research Scholar	Research Guide
Ramesh D. Kolhe	Dr R. M. Mulani